The role of MHC class I glycoproteins in the regulation of induction of cell death in immunocytes by malignant melanoma cells

Daniel Fishman*[†], Brodyanski Irena*[†], Sigal Kellman-Pressman*, Michael Karas[‡], and Shraga Segal*[§]

*Department of Microbiology and Immunology, Faculty of Health Sciences, Ben-Gurion University of the Negev, B.G.U. Cancer Research Center, P.O. Box 653, Beer-Sheva 84105, Israel; and [‡]Diabetes Branch, National Institutes of Health, Bethesda, MD 20892-1770

A deranged expression of MHC class I glycoproteins, characteristic of a variety of malignancies, contributes to the ability of cancer to avoid destruction by T cell-mediated immunity. An abrogation of the metastatic capacity of B16 melanoma cells has been achieved by transfecting an MHC class I-encoding vector into class I-deficient B16 melanoma clones [Gorelik, E., Kim, M., Duty, L. & Galili, U. (1993) *Clin. Exp. Metastasis* 11, 439–452]. We report here that the deranged expression of class I molecules by B16 melanoma cells is more than a mere acquisition of the capacity to escape immune recognition. Namely, cells of the B16 melanoma prompted splenic lymphocytes to commit death after coculture. However, a class I-expressing and nonmetastatic CL8-2 clone was found to be less potent as an inducer of apoptosis than class I-deficient and metastatic BL9 and BL12 clones. Both Thy1.2^{+} and Thy1.2^{−} splenocytes underwent cell death when exposed to the class I-deficient BL9 clone. A proportion of CD4^{+} and CD8^{+} cells among splenocytes exposed to the BL9 clone was lower than that observed in a coculture with cells of the CL8-2 clone. Consistently, none of the melanoma clones studied produced a ligand to the FAS receptor (FAS-L). Thus, our results provide evidence that (*i*) the production of FAS-L may not be the sole mechanism by which malignant cells induce apoptosis in immunocytes, and (*ii*) absence of MHC class I glycoproteins plays an important role in preventing the elimination of potential effector immunocytes by tumor cells.

MHC class I-mediated presentation of antigenic peptides to receptors of cytotoxic T cells plays a key role in the regulation of induction of an immune response against virally infected cells and cells altered by malignant transformation (1). It is not surprising that a deficiency in or an altered ratio between the distinct MHC class I allele-encoded products (1–4), which are characteristic to a variety of malignancies, is of relevance for tumor progression and development of metastatic disease (5, 6). Although the role of MHC class I glycoproteins in the immune response is now well understood, the mechanism of how these molecules determinate the malignant and metastatic capacities of a cancerous cell is still obscure. A number of studies have raised the possibility that tumors of both mouse and human origin can evade immune surveillance actively by delivering apoptotic death signals to lymphocytes in a mode similar to that of cells located in "immune-privileged" sites such as the retina, thyroid gland, etc. (7). It has been reported that some malignant cells constantly synthesize a FAS-L (7, 8).

Gorelik *et al.* (9) have demonstrated that the transfection of H-2K murine MHC class I vector into MHC class I- and class II-deficient B16 BL6-BL8 melanoma cells completely abrogates the capacity of these cells to form metastases when injected into mice. Our present findings provide evidence that in contrast to highly metastatic and MHC-deficient B16 BL6-BL8 melanoma cells, cells that have been transfected with an H2-K class I- (but not with H-2IA class II-) encoding vector exhibited a diminished capacity to induce cell death in murine splenocytes after coculture. These results may shed new light on the role of MHC class I glycoproteins in determining the malignant and metastatic capacity of tumor cells, and may explain why a selective pressure for the loss of expression of these particular antigen-presenting glycoproteins is exerted against the expression of H2-K region-encoded MHC class I glycoproteins in various tumor cells.

Materials and Methods

Cells. The clones of B16 melanoma clones used in this study (the highly metastatic and H-2K[b] and H-2AI-deficient BL9 clone, the highly metastatic H-2K[b]-deficient and H-2IA[k]-positive BL12 clone, and the nonmetastatic H-2K[b]-positive and H-2IA[k]-deficient CL8-2 clone) have been developed (9) and kindly provided by E. Gorelik (Cancer Institute and Department of Pathology, University of Pittsburgh, Pittsburgh). In our laboratory, cells were grown in DMEM (Life Technologies, Paisley, Scotland; GmbH, Karslruhe, Germany) supplemented with 10% (vol/vol) of FCS (Biological Industries, Beit Haemek, Israel). We ascertained the phenotypic stability of the clones by the *in vivo* examination of their metastatic capacities in syngeneic C57BL/6 mice and by staining the cells with the Y-3 hybridoma-derived anti-H-2K murine class I antibodies.

Coculture of Melanoma Cells with Murine Splenocytes. CL8-2, BL9, and BL12 melanoma cell lines were grown in tissue-culture dishes (Corning) to subconfluent monolayers in DMEM. Spleens were excised surgically from 5-week-old syngeneic C57BL/6 mice. Then the capsule of one pole of each spleen was dissected, a 21-gauge needle was inserted into the other pole, and splenocytes were flushed gently out from the spleens by using a syringe and DMEM. Splenocytes were counted by light microscopy and added to the cultures of tumor cells in splenocyte/melanoma-cell ratios of 10:1 (usually, 8×10^6 to 10×10^6:0.8×10^6 to 1×10^6). In some experiments, the water-soluble membrane-permeable caspase inhibitor Y-VAD (Calbiochem–Nova Biochem) was added to culture medium. After different incubation intervals, nonadherent cells were collected by gentle shaking of culture dishes, harvesting, and centrifugation at $400 \times g$ for 10 min at 4°C. Viability of nonadherent splenocytes was

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assessed by mixing cell suspension with an equal volume of 0.1% of trypan blue in PBS and examination by light microscopy.

Analysis of Apoptosis by Staining with Propidium Iodide and Flow Cytometry. After a given period of coincubation with melanoma cells, splenocytes were harvested, washed twice with ice-cold PBS containing 1% BSA (fraction V; Sigma) (PBS/BSA) by centrifugation at $250 \times g$ for 5 min at 4°C, and fixated in 70% ethanol with propidium iodide. The DNA content of the cells was analyzed by using a FACStar flow cytometer (Becton Dickinson). Separation between T and non-T cells has been achieved by using Dynabeads mouse PanT (Thy1.2) and the Detachabead system (Dynal, Oslo) according to the protocol supplied by the manufacturer.

DNA Fragmentation Assay. After coculture, cells were harvested, washed twice with ice-cold PBS by centrifugation, and counted. Then 3×10^6 cells were resuspended in a buffer containing 10 mM Tris·HCl (pH 8.0), 5 mM EDTA (pH 8.0), and 50 mM NaCl and incubated on ice for 20 min. Cells were passed several times through a 21-gauge needle, and cell debris was sedimented by centrifugation at $12,000 \times g$ for 15 min at 4°C. Clear lysates were extracted twice with phenol/chloroform, and DNA was precipitated by the addition of 2 volumes of cold ethanol and potassium acetate to a final concentration of 0.3 M. DNA precipitates were redissolved in a buffer containing 10 mM Tris·HCl (pH 8.0), 1 mM EDTA (pH 8.0), and 0.5 mg/ml of DNase-free RNase (Sigma), incubated for 2 h at 37°C, and then subjected to electrophoresis in 2% agarose gel.

Detection of the Expression of Phosphatidyl Serine in Splenocytes by Staining with Annexin V. Membrane expression of phosphatidyl serine in splenocytes exposed to melanoma cells has been examined by staining nonfixed spleen cells collected from the cocultures with FITC-conjugated annexin V by using a commercial kit according to the protocol provided by the manufacturer (Roche Molecular Biochemicals) and by flow cytometry. Necrotic cells were excluded from the analysis by counterstaining with propidium iodide and gating on propidium iodide-negative cells.

Immunoblotting. Cells were washed with ice-cold PBS and lysed in boiling lysis buffer containing 10 mM Tris·HCl (pH 8.0) and 1% SDS. After additional boiling for 5 min, lysates were passed several times through a 21-gauge needle and cleared by centrifugation at $12,000 \times g$ for 5 min. Protein samples containing equal amounts of protein (50 µg) were separated by 10% SDS/PAGE and subjected to immunoblotting with appropriate anti-FAS M-20 (Santa Cruz Biotechnology) or anti-FAS-L (PharMingen) antibodies. Immunoreactive proteins were visualized by using an enhanced chemiluminescence kit (Amersham Pharmacia).

Reverse Transcription–PCR Detection of Expression of FAS-L mRNA. Total RNA was isolated from splenocytes obtained from C57BL/6 mice (used as controls) and from melanoma cells using Trireagent (MRS, Cincinnati). The total RNA then was treated with RNase-free DNase, reverse transcribed to cDNA, and amplified by PCR, generating a fragment of FAS-L [forward primer, 5′-CACTCAAGGTCCATCCCTCTG-3′; reverse primer, 5′-TAGCTGACCTGTTGGACCTTGG-3′ (10)] or a fragment of actin (forward primer, 5′-GACGAGGCCCAGAG-CAAGAG-3′; reverse primer, 5′-GGGCCGGACTCATCG-TACTC-3′). The following conditions were used: 1 cycle at 94°C for 5 min, 30 cycles at 94°C for 1 min, 55°C for 2 min, and 72°C for 2 min. The PCR products were resolved in a 2% agarose.

Analysis of Membrane Expression of Lymphocyte Differentiation Markers by Flow Cytometry. After coculture with melanoma cells, splenocytes were washed twice with ice-cold PBS/BSA, incu-

bated with anti-CD3, -CD4, -CD8, -B220 (Roche Molecular Biochemicals) and -NK1.1 (PharMingen) monoclonal antibodies for 60 min at 4°C and with the corresponding FITC-conjugated secondary antibody (Jackson ImmunoResearch) for an additional 60 min at 4°C in the dark. After two additional washes, cells were resuspended and fixated in 2% paraformaldehyde in PBS (pH 7.4) before FACS analysis. FACS analysis was performed by using a FACStar flow fluorocytometer (Becton Dickinson).

Results

Expression of H-2K^b Allele-Encoded MHC Class I but Not H-2IA^k Allele-Encoded MHC Class II Glycoproteins Abrogates the Capacity of Melanoma Cells to Induce Apoptosis in Lymphocytes. Having observed the induction of apoptosis in splenocytes cocultured with the metastatic MHC class I- and class II-deficient B16BL6-BL8 melanoma cells (data not shown), experiments were performed to assess the cytotoxic capacity of these cells transfected with either H-2K^b- (CL8-2 clone) or H-2IA^k- (BL12 clone) encoding vectors or with the original control vector containing the *neo* resistance gene alone (BL9 clone). As depicted in Fig. 1*A*, splenocytes coincubated with BL9 or BL-12 cells for 24, 48, and 72 h were characterized by fractions of 38, 18, and 14% (for BL9) and 34, 28, and 22% (for BL12) of nonviable cells, respectively, than those exposed to CL8-2 cells (90, 70, and 29% for 24, 48, and 72 h, respectively), as assessed by trypan blue-dye exclusion. Splenocytes cocultured with melanoma cells also were examined for the presence of cells with a sub-G$_1$ DNA content by flow cytometry of fixed cells stained by propidium iodide. The percentage of lymphocytes with a sub-G$_1$ DNA content found in cocultures with class I-deficient melanoma cells (55 and 44% in cocultures with BL9 and BL12 cells, respectively) was about two times higher than that detected in cocultures with the class I-positive CL8-2 melanoma clone (28% following cocultures for 48 h; Fig. 1*B*). Consistently, the enhanced capacity to induce apoptosis in splenocytes has been exhibited also by other B16BL6 melanoma clones that did not express H-2K^b MHC class I glycoproteins (data not shown). No cells with a sub-G$_1$ DNA content were detected among freshly isolated splenocytes used as a control (data not shown). The characteristic pattern of internucleosomal DNA fragmentation in splenocytes cocultured with both BL9 and CL8-2 cells appeared as early as several hours after coincubation, implying that this assay does not provide quantitative but rather qualitative data in relation to apoptotic death (Fig. 1*C*). It is noteworthy, however, that the fragmentation of DNA appeared in splenocytes exposed to BL9 cells earlier (3 h of coculture; Fig. 1*C*, lane 9) than in those incubated with CL8-2 cells (6 h of coculture; Fig. 1*C*, lane 12). The induction of the apoptotic ladder formation in splenocytes by cocultured melanoma cells was diminished significantly by employing the membrane-permeable inhibitor of caspase-1, namely YVAD-CHO, in coculture experiments (Fig. 1*C*, lanes 10 and 11), which suggested the activation of apoptotic proteases after the induction of death signals in splenocytes by melanoma cells. The capacity of H-2K-negative melanoma cells to induce apoptosis in splenocytes was confirmed also by the assessment of membrane expression of phosphatidyl serine by the latter cells, as determined by using FITC conjugated by annexin V. As depicted in Fig. 1*D*, the percentage of annexin V-positive splenocytes found in cocultures with BL9 cells was higher (30%) than that detected in cocultures with CL8-2 cells (16.5%) after 3 h of the coculture.

The Capacity of H-2K^b-Negative Melanoma Cells to Induce Apoptosis in Lymphocytes Does Not Correlate with the Expression of FAS-L by These Cells. To test whether the expression of H-2K^b attenuated the transmission of a death signal by melanoma cells to spleen
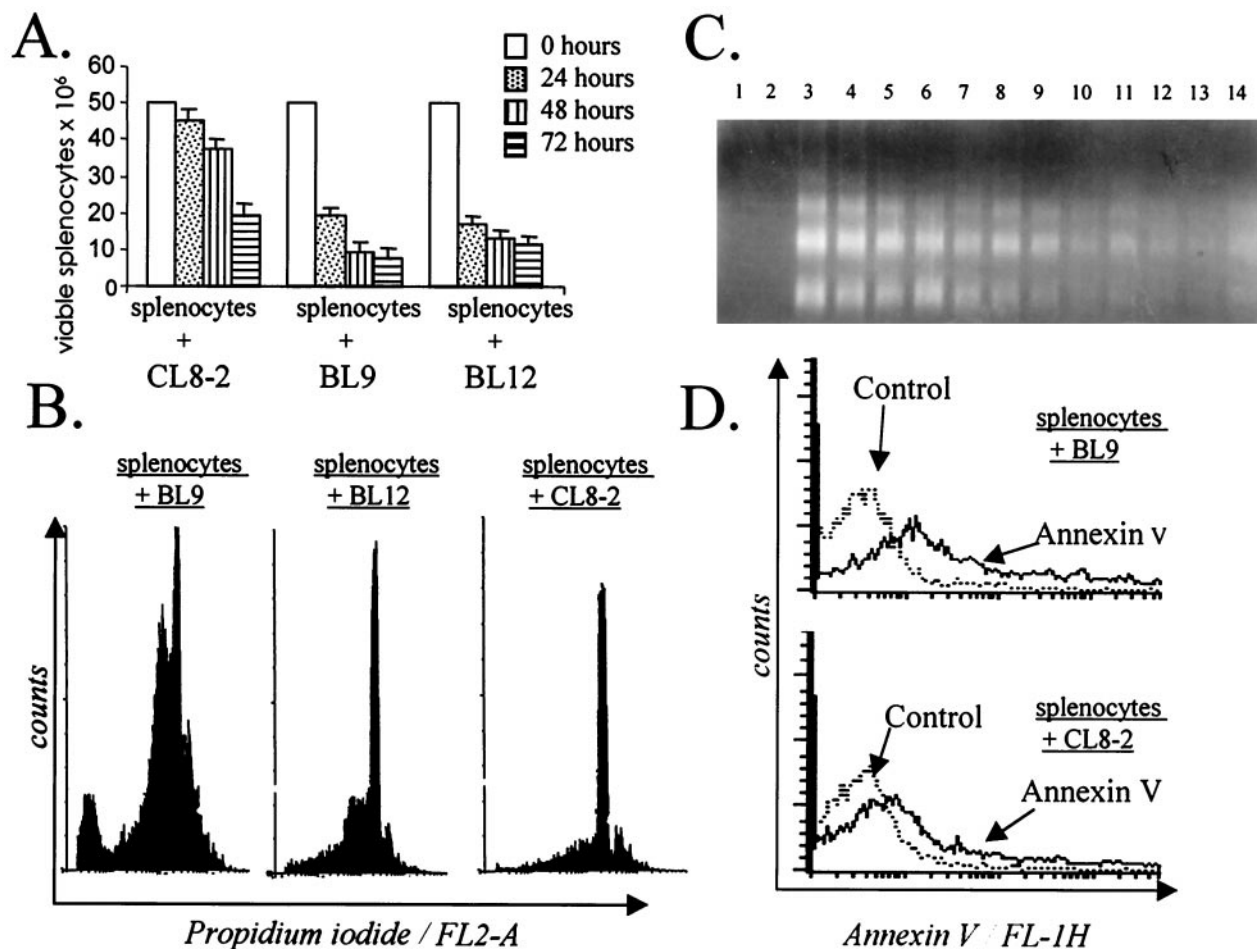
Fig. 1. Cell death in spleen cells after coculture with MHC class I-deficient melanoma cells. (*A*) Splenocytes were cocultured with BL9, BL12, and CL8-2 clones for the indicated times and harvested, and their viability mean $\pm$ SEM by trypan blue is shown as the vertical axis. The results are one of 10 independent experiments. (*B*) Splenocytes were exposed to either BL8, BL12, or CL8-2 cells for 48 h, and propidium-iodide analysis by flow cytometry is shown. The results are one of 10 independent experiments. (*C*) Splenocytes were exposed to either BL9 or CL8-2 cells for 3 or 6 h and analyzed for DNA fragmentation. Lanes 1, 3, 4, 5, 9, 10, and 11 are splenocytes cocultured with BL9 cells; lanes 2, 6, 7, 8, 12, 13, and 14 are splenocytes cocultured with CL8-2 cells; lanes 1 and 2 are cocultured for 5 min; lanes 3, 4, 5, 6, 7, and 8 are cocultured for 6 h; and lanes 9, 10, 11, 12, 13, and 14 are cocultured for 3 h. Y-VAD has been used in the following concentrations: lanes 4, 7, 10, and 13, 100 μM; lanes 5, 6, 11, and 14, 50 μM. The results are one of three independent experiments. (*D*) Splenocytes were exposed to either BL9 or CL8-2 cells for 3 h and examined for membrane expression of phosphatidyl serine by staining with FITC-conjugated annexin V. Necrotic cells were excluded from the analysis by staining with propidium iodide and gating on propidium-iodide-negative cells. The results are one of three independent experiments.

cells via the FAS-L/FAS-receptor system, we examined the expression of both the FAS receptor and its ligand on splenocytes and melanoma cells. As depicted in Fig. 2*A* (*Left*), the expression of the FAS receptor that was barely detectable in freshly isolated splenocytes became enhanced significantly during coincubation with either BL9 or CL8-2 cells. It is noteworthy that the increase in FAS-receptor expression was significantly higher in splenocytes incubated with CL8-2 cells (Fig. 2*A*, *Left*); i.e., the up-regulation of FAS did not correspond to the extent of killing of splenocytes by melanoma cells. Both melanoma clones were characterized by the same level of FAS expression (Fig. 2*A*, *Right*).

The expression of FAS-L in melanoma cells has been analyzed on the mRNA level by using reverse transcription—PCR (Fig. 2*B*, *Left*) and on the protein level by immunoblotting (Fig. 2*B*, *Right*). None of the melanoma clones examined was characterized by the expression of FAS-L. Thus, a constitutive production of FAS-L by tumor cells (8, 10) does not seem to be a sole mechanism by which malignant melanoma cells may induce apoptotic death in immunocytes.

H-2K[b]-Negative BL9 Cells Deplete Different Subsets of Lymphocytes from a Mixed Population of Spleen Cells. To define the particular subset(s) of splenocytes that was triggered to commit cell death by MHC class I-negative melanoma cells after coculture with melanoma cells, splenocytes were fractionated according to the expression of the Thy1.2 epitope. Each fraction of cells (Thy1.2[+] or Thy1.2[-]) was analyzed for the cellular DNA content by flow cytometry. Both Thy1.2[+] and Thy1.2^{-} cells display a significant fraction of cells with sub-G_1 DNA content ($\approx$70%) after 72 h of a coculture with BL9 melanoma cells (Fig. 3*A*). No cells with a low DNA content were detected among splenocytes incubated with CL8-2 melanoma cells. After coculture with either of the aforementioned melanoma clones, splenocytes were analyzed also for the expression of membrane-associated differentiation molecules characteristic to the specific subpopulations of splenocytes. The percentage of CD4^{+} and CD8^{+} cells in a population of splenocytes exposed to BL9 cells (14.4 and 21.3%, respectively) was lower than that detected in a coculture with CL8-2 cells (29.5 and 41% for CD4^{+} cells and CD8^{+} cells, respectively; Fig. 3*B*). In contrast, splenocytes exposed to BL9 melanoma cells
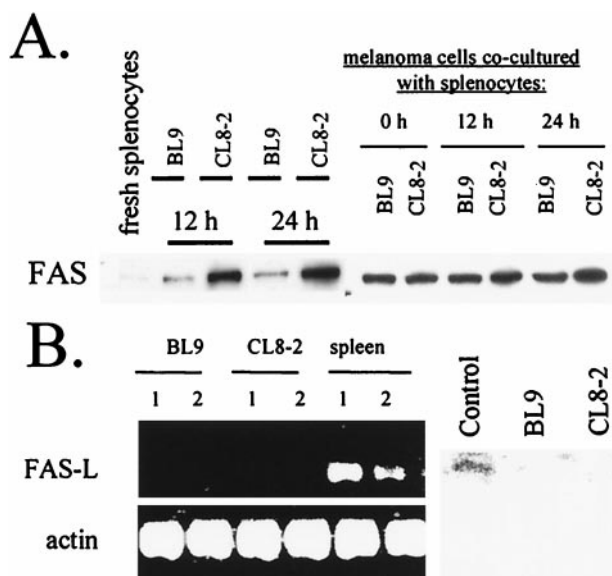
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Fig. 2. The expression of FAS and FAS-L in splenocytes and melanoma cells. (*A*) Protein lysates prepared from either spleen (*Left*) or melanoma (*Right*) cells were subjected to immunoblotting with anti-FAS antibodies. The results are one representative of three independent experiments. (*B*) RNA samples isolated from BL9 and CL8-2 cells as well as from freshly isolated splenocytes in duplicates (1 and 2) were subjected to reverse transcription—PCR. The results are one of three independent experiments (*Left*). Protein lysates were prepared from melanoma clones and subjected to immunoblotting with anti-FAS-L antibodies. Splenocytes exposed to 600 units/ml of IFN-γ for 24 h were used as a positive control. The results are one of two independent experiments (*Right*).

displayed a fraction of 17% of cells expressing the NK1.1 receptor, which was barely detectable among splenocytes exposed to CL8-2 cells (2.16%). Other surface molecules, such as TCR, CD3, and B220, were expressed at equal levels in spleen cells cocultivated with either BL9 or CL8-2 cells. Based on these findings, we hypothesize that H-2K^b-negative BL9 cells (but not H-2K^b-positive CL8-2 cells) may deplete CD4$^+$ and CD8$^+$ cells selectively from a mixed population of splenocytes and activate an NK1.1 subset of cells.

Discussion

Metastatic cells are characterized often by a stable expression of an immune escape phenotype. This phenotype is associated frequently with alterations in the MHC class I-related antigen-processing and -presenting machinery that enable tumors to alter the exposure of tumor-specific antigens and thereby escape immune surveillance (7). Our present findings provide evidence that the latter derangement may lead also to more than a mere acquisition of the capacity to escape immune recognition and activation of immune effector-killer cells. Namely, highly metastatic MHC class I- and class II-negative B16BL6-BL8 malignant melanoma cells were found to eliminate the attacking immunocytes. This capacity to induce cell death is associated with the loss of expression of MHC class I glycoproteins, because the transfections of an H-2K^b murine MHC class I glycoprotein-encoding expression vector into BL8 cells (CL8-2 clone) has been found to attenuate death signals delivered by malignant cells significantly, a phenomenon which has not been observed in BL8 cells transfected with the H-2IA^k murine class II glycoprotein-encoding vector (BL12 clone) or the expression vector alone (BL9 clone) (Fig. 1). The fact that the expression of H-2K^b region-encoded glycoproteins diminishes the delivery of apoptotic signals to splenic lymphocytes by malignant cells is consistent with our previous findings (11–14) that the reexpres-
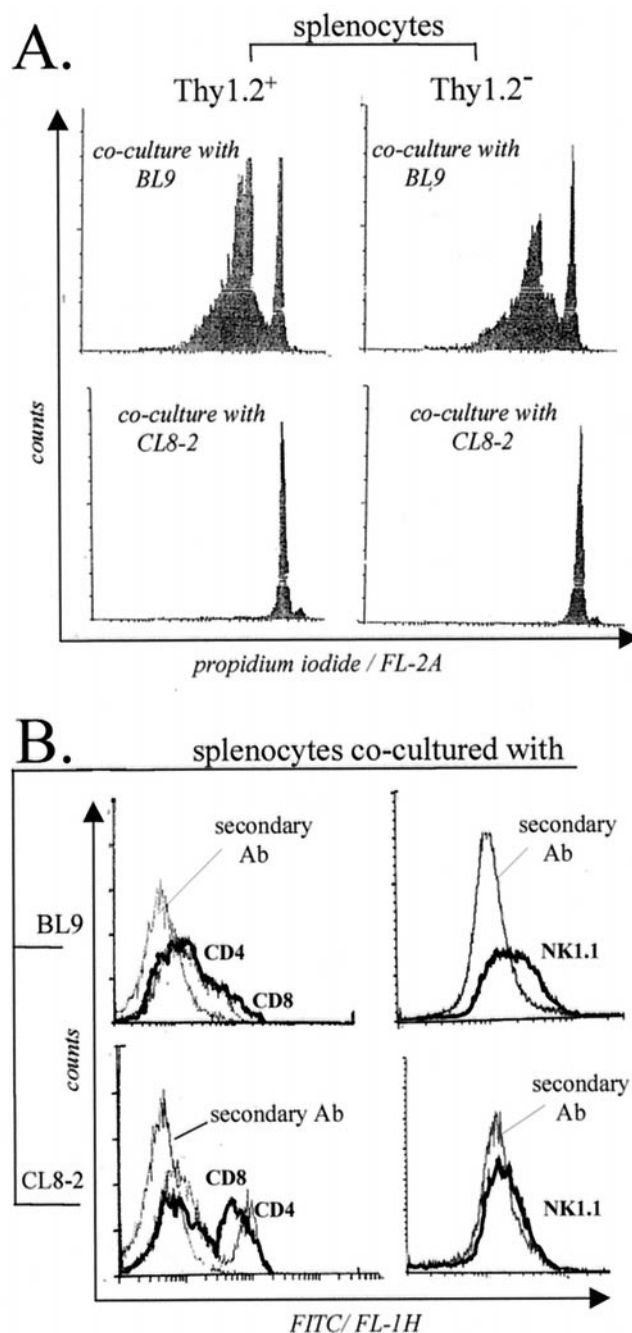

Fig. 3. H-2K-deficient melanoma cells deplete distinct subsets of splenocytes from a mixed spleen-cell population after coculture. (*A*) Splenocytes were cocultured for 72 h with either BL9 or CL8-2 melanoma cells. Lymphocytes then were harvested and fractionated to Thy1.2$^+$ and Thy1.2$^-$ cells. Each fraction was subjected to the analysis of cellular DNA content by flow cytometry. The results represent one of two independent experiments. (*B*) After coculture of splenocytes with melanoma cells for 72 h, splenocytes were harvested, and the expression of CD4 (thin curve), CD8 (bold curve), and NK1.1 (bold curve) was analyzed by flow cytometry by using appropriate antibodies. In the upper left, CD4 and CD8 curves are overlapping. The results are one representative of three independent experiments.

sion of H-2K^b-encoded glycoproteins by H-2K-deficient tumor cells of different origin confers high immunogenicity and aborts metastatic capacity.

The precise mechanism by which MHC class I regulates the delivery of apoptotic signals to lymphocytes remains unknown.

According to our data, the expression of H-2K^b does not attenuate the transmission of death stimuli through the FAS-L/FAS module. First, no correlation between the expression of the FAS receptor and the extent of cell death has been observed in splenocytes (Fig. 2A). Second, none of the melanoma clones presently investigated constitutively produce FAS-L (Fig. 2B). The latter observation does not correspond to previously reported enhanced production of FAS-L by melanoma cells (10). A possible explanation for such contradiction may be provided by Chappel *et al.* (15), who also were unable to find FAS-L in randomly sampled melanoma lines. It has been reasoned that in many cases the detection of FAS-L in malignancies has been based on staining cells with low-specificity anti-FAS-L C-20 antibodies or may stem from the contamination of RNA preparations, which were subjected for reverse transcription—PCR, with genomic DNA (16, 17). Some reports implicate molecules other than FAS-L in tumor-mediated "counter-attack" phenomenon. For example, the TNF-related apoptosis-inducing ligand expressed by immune-privileged and malignant cells may be capable also of killing lymphoid cells under certain conditions (18, 19). Several tumor-associated antigens expressed by malignant cells also may induce apoptosis in activated T cells (20, 21). We cannot exclude a possibility that the presence of H-2K-encoded glycoproteins on the plasma membrane of melanoma cells may provide a rescue signal to lymphocytes triggered to commit cell death by a proapoptotic molecule(s) produced by tumor cells. Recent findings of Zaks *et al.* (22) have challenged the mechanistic view of tumor counter-attack as a simple engagement of a receptor expressed on lymphoid cells by a FAS-L produced by a malignant cell. Tumor-reactive T cells were found to undergo apoptosis after exposure to the cognate tumor antigen where FAS-L was not expressed by tumor cells but rather by a proportion of T cells themselves.

In the present study, we characterized the subset(s) of lymphocytes which are prompted to commit cell death after exposure to MHC class I-negative melanoma cells. We found that both T and non-T cells undergo cell death when cocultured with MHC class I-negative BL9 cells (Fig. 3A). To the best of our knowledge, one report, which describes killing of non-T cells by a malignant tumor, has been published (23). Splenocytes exposed to BL9 cells were characterized by a diminished proportion of cells expressing CD4 and CD8 coreceptors as compared with those cocultured with CL8-2 cells (Fig. 3B). In contrast, none of the splenocytes exposed to CL8-2 cells were character-ized by the expression of the NK1.1 receptor, whereas a significant fraction of NK1.1$^+$ cells was found among splenocytes cocultured with the BL9 clone (Fig. 3B). NK1.1 is a C type lectin receptor characteristic of natural killer and NK1.1$^+$ T cells (24), whose effector function, such as cytolysis and secretion of cytokines (25), may be modulated in part after the ligation of NK1.1 by a yet-undefined ligand (26). Other essential receptors that regulate the function of NK1.1$^+$ cells, namely killer inhibitory receptors, recognize MHC class I and thereby transduce to the latter cells potent inhibitory signals (27). Hence, the existence of a balance between the expression of a ligand matching activating NK1.1 and other receptors on one hand, and the expression of inhibitory MHC class I molecules on the other hand, by a putative target cell may play a determinative role in the activation or inhibition of the NK1.1$^+$ cells (28). Therefore, it was not surprising to find NK1.1$^+$ cells among splenocytes exposed to MHC class I-negative BL9 cells (but not to class I-positive CL8-2 cells). Moreover, our present finding may be explained on the basis of an alternative and indirect killing scenario, namely MHC H-2K^b class I-negative melanoma cells selectively activate a distinct population of splenocytes (for example, NK cells); these cells turn their "apoptotic guns" against other subsets of splenocytes. This assumption is supported further by previous findings implicating NK1.1$^+$ cells in the suppression of the immune response and hemopoiesis because of their cytolytic activity and potential to secrete large amounts of Th1 inhibitory cytokines (28, 29).

In conclusion, our results explain a mechanism not previously described in tumor immunology. Namely, the derangements in MHC class I expression by a malignant tumor may contribute to defensive as well as offensive strategies used by highly metastatic tumor cells to evade immune surveillance. The further elucidation of the mechanisms by which MHC class I molecules attenuate death signals delivered by tumor cells when attacking immunocytes is important for the understanding of basic immunological events as well as for the prevention of metastatic spread.

1. Townsend, A. & Bodmer, H. (1989) *Annu. Rev. Immunol.* **7,** 601–624.
2. De Batselier, P., Katzav, S., Gorelik, E., Feldman, M. & Segal, S. (1980) *Nature (London)* **288,** 179–181.
3. Katzav, S., De Batselier, P., Tartakovsky, B., Feldman, M. & Segal, S. (1983) *J. Natl. Cancer Inst.* **71,** 317–324.
4. Tanaka, K., Gorelik, R., Nozumi, N. & Jay, G. (1988) *Mol. Cell. Biol.* **8,** 1857–1861.
5. Algarra, I., Gaforio, J. J., Cabrera, T., Collado, A. & Garrido, F. (1999) *J. Biol. Regul. Homeost. Agents* **13,** 90–96.
6. Gilboa, E. (1999) *Cancer Immunol. Immunother* **48,** 382–385.
7. O'Connell, J., Bennett, M. W., O'Sullivan, G. C., Collins, J. K. & Shanahan, F. (1999) *Immunol. Today* **20,** 46–52.
8. O'Connell, J., Bennett, M. W., O'Sullivan, G. C., Collins, J. K. & Shanahan, F. (1999) *Dis. Esophagus* **12,** 83–89.
9. Gorelik, E., Kim, M., Duty, L. & Galili, U. (1993) *Clin. Exp. Metastasis* **11,** 439–452.
10. Hahne, M., Rimoldi, D., Schroter, M., Romero, P., Schreier, M., French, L. E., Schneider, P., Bornand, T., Fontana, A., Lienard, D., *et al.* (1996) *Science* **274,** 1363–1366.
11. Gopas, J., Segal., S., Hammerling, G., Bar-Eli, M. & Rager-Zisman, B. (1989) *Immunol. Lett.* **17,** 261–265.
12. Isakov, N., Katzav, S., Feldman, M. & Segal, S. (1983) *J. Natl. Cancer Inst.* **71,** 139–145.
13. Eisenbach, L., Segal, S. & Feldman, M. (1983) *Int. J. Cancer* **32,** 113–120.
14. Har-Vardi, I., Gopas, J., Rager-Zisman, B., Hammerling, G. J., Segal, S. & Aboud, M. (1992) *Invasion Metastasis* **12,** 301–308.
15. Chappel, D. B., Zaks, T. Z., Rosenberg, S. A. & Restifo, P. (1999) *Cancer Res.* **59,** 59–62.
16. Smith, D., Sieg, S. & Kaplan, D. (1998) *J. Immunol.* **160,** 4159–4160.
17. Restifo, N. P. (2000) *Nat. Med.* **6,** 493–495.
18. Giovarelli, M., Musiani, P., Garotta, G., Ebner, R., Di Carlo, E., Kim, Y., Cappello, P., Rigamonti, L., Bernabei, P., Novelli, F., *et al.* (1999) *J. Immunol.* **163,** 4886–4893.
19. Bretz, J. D., Rymaszewski, M., Arscott, P. L., Myc, A., Ain, K. B., Thompson, N. W. & Baker, J. R. (1999) *J. Biol. Chem.* **274,** 23627–23632.
20. Gimmi, C. D., Morrison, B. W., Mainprice, B. A., Gribben, J. G., Boussiotis, V. A., Freeman, G. J., Park, S. Y., Watanabe, M., Gong, J., Hayes, D. F., *et al.* (1996) *Nat. Med.* **2,** 1367–1370.
21. Nakashima, M., Sonoda, K. & Watanabe, T. (1999) *Nat. Med.* **5,** 938–942.
22. Zaks, T. Z., Chappell, D. B., Rosenberg, S. A. & Restifo, N. P. (1999) *J. Immunol.* **162,** 3273–3279.
23. Khar, A., Varalakshmi, C., Pardhasardhi, B. V. V., Mubarak, A. & Kumari, A. L. (1998) *Cell. Immunol.* **189,** 85–91.
24. MacDonald, H. R. (1995) *J. Exp. Med.* **182,** 633–638.
25. Kawamura, T., Seki, S. S., Takeda, K., Narita, J., Ebe, Y., Naito, M., Hiraide, H. & Abo, T. (1999) *Cell. Immunol.* **193,** 219–225.
26. Carlyle, J. R., Martin, A., Mehra, A., Attisino, L., Tsui, F. I. & Zuniga-Pflucker, J. C. (1999) *J. Immunol.* **162,** 5917–5933.
27. Lanier, L. L. (1998) *Annu. Rev. Immunol.* **16,** 359–393.
28. Yu, Y. Y. L., Kumar, V. & Bennet, M. (1992) *Annu. Rev. Immunol.* **10,** 189–213.
29. Kikly, K. & Dennert, G. (1992) *J. Immunol.* **149,** 3489–3493.